Expanded View Figures

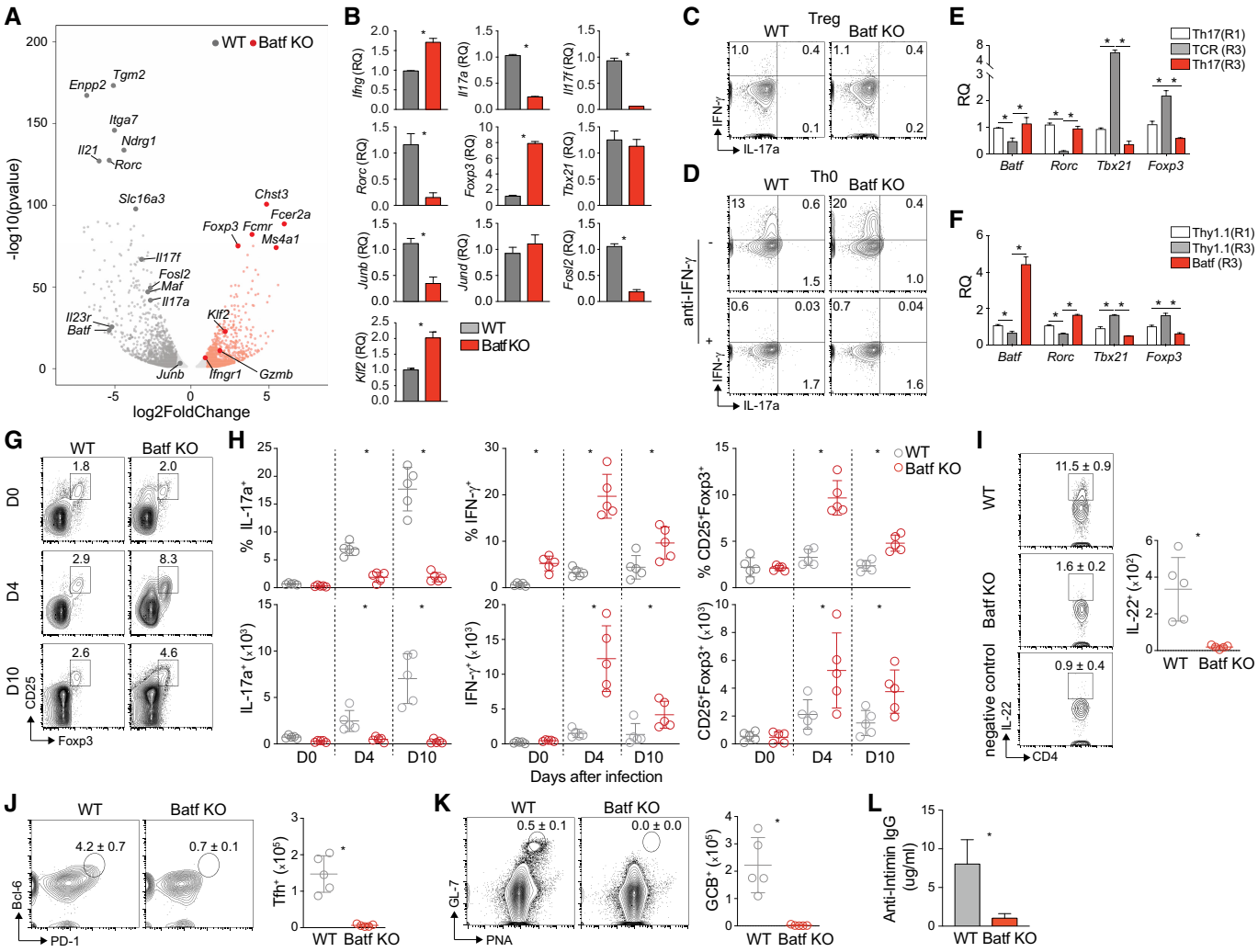

Figure EV1.

Figure EV1. Batf deficiency skews CD4 T cell development toward Th1/Treg phenotypes resulting in altered response to *Citrobacter rodentium* infection.

A Naÿve WT and Batf KO $CD4^+CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions. On day 5, total RNA was extracted from resting Th17 cells and used for RNA-seq. Differential gene expression (fold change > 2, fdr < 0.05) was represented as a volcano plot.

B Naÿve WT and Batf KO $CD4^+CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions for 5 days. Total RNA was extracted from resting or restimulated (6 h with anti-CD3) cells and used for gene expression analysis by RT–PCR (resting: *Rorc*, *Foxp3*, *Tbx21*, *Junb*, *Jund*, *Fosl2*, and *Klf2*; restimulated: *Ifng*, *Il17a*, and *Il17f*).

C, D Day 5 of differentiated WT and Batf KO cells cultured under Treg (C) or Th0 conditions (D, cultured with or without anti-IFN-γ) were restimulated with PMA and ionomycin for 5 h, and stained intracellularly for IFN-γ and IL-17a expression.

E Naÿve $CD4^+CD62L^{hi}$ T cells from IL-17a reporter mice ($Il17a^{eGFP}$) were cultured under Th17-polarizing conditions. On day 5, $eGFP^+$/IL-$17a^+$ cells were sorted and cultured for two additional rounds in the presence of anti-CD3 alone (TCR(R3)) or Th17-polarizing cytokines (Th17(R3)). Cells from round 1 (Th17(R1)) and round 3 (TCR(R3) and Th17(R3)) were used for total RNA isolation to assess gene expression by RT–PCR (data normalized to Th17(R1)).

F Naÿve $CD4^+CD62L^{hi}$ T cells from IL-17a reporter mice ($Il17a^{eGFP}$) were cultured under Th17-polarizing conditions. On day 2, cells were transduced with control (Empty) or retrovirus expressing Batf-Thy1.1 (Batf). $eGFP^+$/IL-$17a^+$$Thy1.1^+$ cells were sorted on day 5 and cultured for two additional rounds in the presence of anti-CD3 alone. $Thy1.1^+$ cells from round 1 (Thy1.1(R1)) and round 3 (Thy1.1(R3) and Batf(R3)) were used for total RNA isolation to assess gene expression by RT–PCR (data normalized to Thy1.1(R1)).

G, H WT and Batf KO mice were infected by gavage with 2×10^9 cfu/ml *Citrobacter rodentium* (*C.r.*). Cells were isolated from the colon, restimulated with PMA and ionomycin, and stained intracellularly for flow cytometric analysis of cytokine production and Foxp3 (G) expression and were presented as percentages of the indicated cell population (H, top) and numbers of positive cells (H, bottom) at the indicated time points.

I Mice were sacrificed on day 10 postinfection. Cells were isolated from the colon tissue, restimulated with PMA and ionomycin, and intracellularly stained for cytokine production. IL-22-producing $CD4^+$ T cells on day 10 postinfection were assessed in the colon of infected WT and Batf KO mice with contour plots showing percentage ± s.e.m. and average of cell numbers. Data are gated on viable $TCR\beta^+$. Unstimulated cells were used as negative controls.

J–L Cells from MLN of WT and Batf KO mice on day 10 postinfection were used for Tfh ($CD4^+CD44^+CXCR5^+PD\text{-}1^+Bcl6^+$) (J) and germinal center B cell ($B220^+PNA^+GL\text{-}7^+$) (K) staining with contour plots showing percentage ± s.e.m. and average of cell numbers. Anti-Intimin IgG antibody was measured using serum of WT and Batf KO mice on day 21 postinfection (L).

Data information: Data are representative of two independent experiments with similar results (A) or mean ± s.e.m. of three independent experiments (B–F). (*$P < 0.05$; two-sided *t*-test). RQ, relative quantification. Data are gated on viable $CD4^+TCR\beta^+$ and mean ± s.e.m. of $n = 5$ mice and representative of two independent experiments with similar results (G–L) (*$P < 0.05$; two-sided *t*-test).

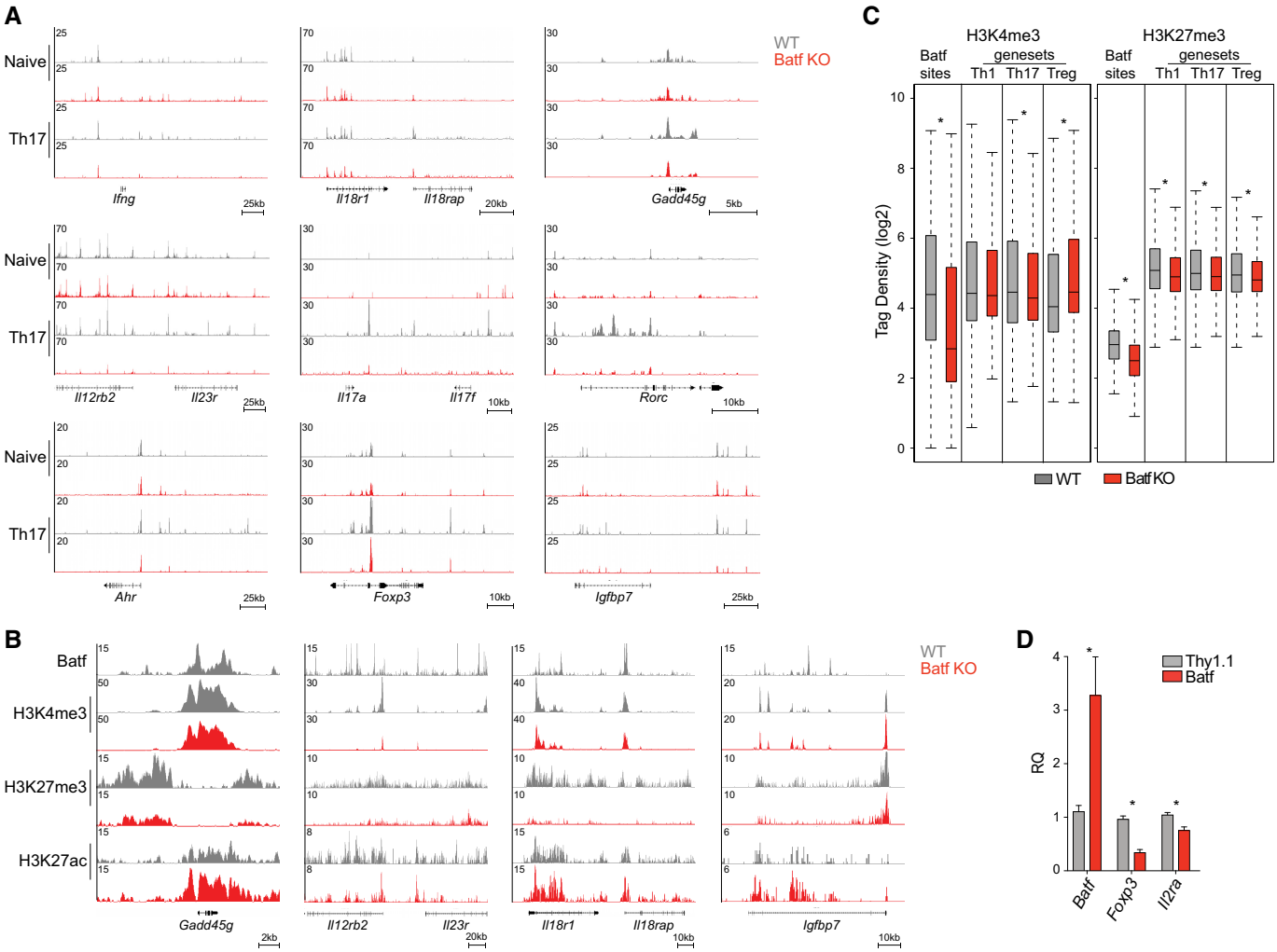

Figure EV2. Batf modulates chromatin landscape in Th17 cells.

A Chromatin accessibility (ATAC-seq) was performed using naÿve or day 2 WT and Batf KO cells cultured under Th17 conditions (data from GSE123209). Tracks visualized using the Integrated Genome Browser (IGB) compare chromatin states of WT and Batf KO Th17 in the indicated gene loci.

B Naÿve CD4^{+}CD62L^{hi} WT and Batf KO T cells cultured under Th17-polarizing conditions for 5 days were assessed for binding of Batf, H3K4me3, H3K27me3, and H3K27ac at selected loci.

C Naÿve WT and Batf KO CD4^{+}CD62L^{hi} T cells were cultured under Th17-polarizing conditions for 5 days and ChIP-seq for transcription factors Batf and histone modification (H3K4me3 and H3K27me3) was performed. Boxplots display normalized tag density of H3K4me3 (left) and H3K27me3 (right) centered around ±1 kb of Batf sites or ± 2.5 kb of peak centers that were annotated to the nearest Th1-, Th17-, and Treg-specific genes (Li *et al*, 2012b; GSE39756) compared between WT and Batf KO Th17-polarized cells. Central bands, boxes, and whiskers show median, upper and lower quartile, and maximum and minimum values, respectively.

D Naÿve WT CD4^{+}CD62L^{hi} T cells were cultured under iTreg polarizing conditions. On day 2, cells were transduced with control (Thy1.1; Empty) or Batf-Thy1.1 retrovirus. Thy1.1^{+} cells were sorted on day 5 and used for total RNA isolation to assess gene expression by RT–PCR (data normalized to Thy1.1).

Data information: Data are representative of two independent experiments with similar results (A–C), *P < 0.05 (Mann–Whitney test); or mean ± s.e.m. of three independent experiments (D) (*P < 0.05; two-sided t-test).

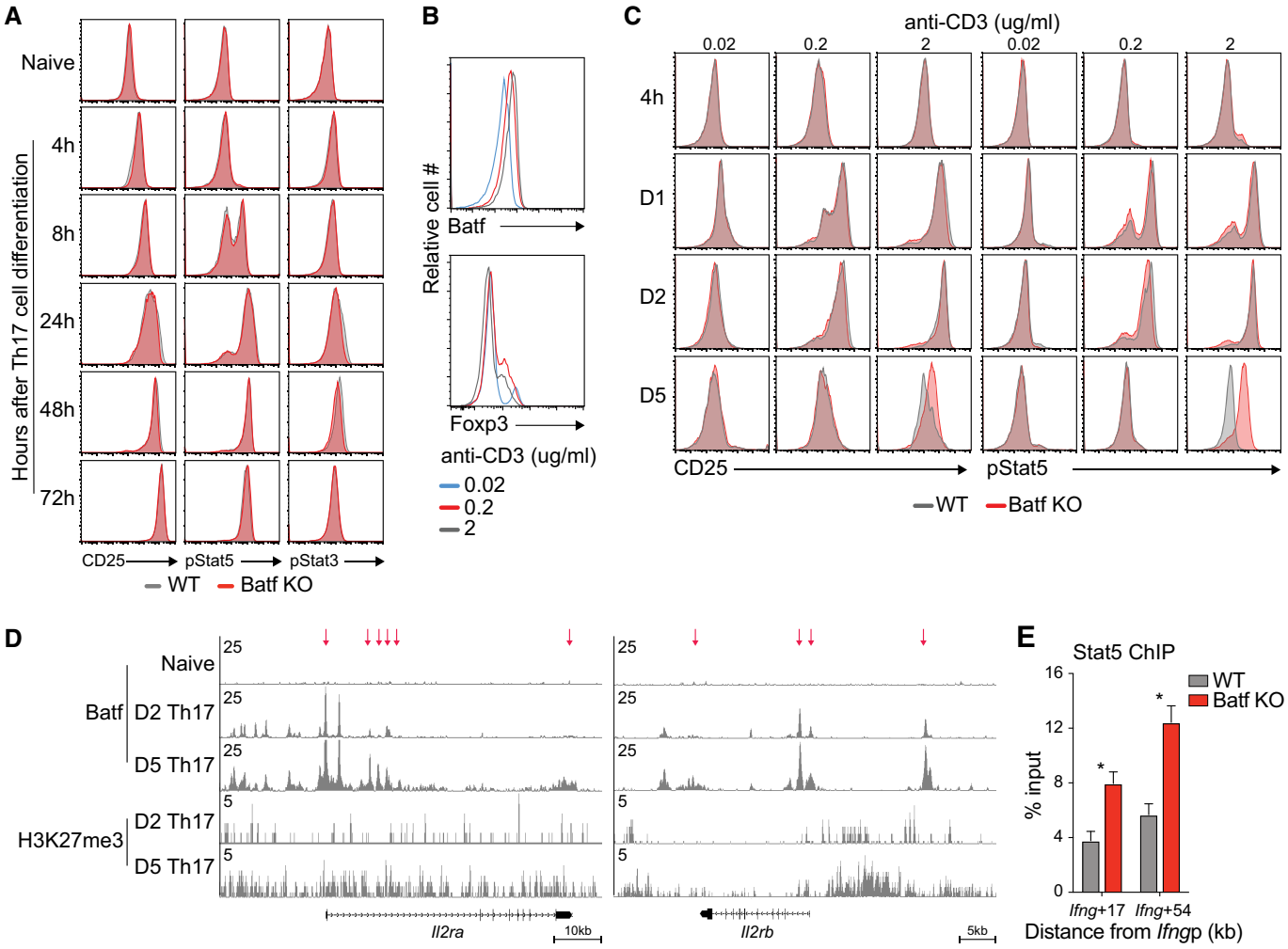

Figure EV3. Batf regulates the *Il2ra* gene expression in the late stage of Th17 cell differentiation.

A–C Naÿve WT and Batf KO $CD4^{+}CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions with 2 μg/ml of anti-CD3 (A) or with increasing amounts of anti-CD3 (B, C). Expression of CD25 expression, phosphorylated Stat5 (pStat5), and phosphorylated Stat3 (pStat3) were assessed by flow cytometry at the indicated time points, (A, C), or on day 5 (B).

D Tracks show Batf and H3K27me3 ChIP-seq data from day 2 (D2) and day 5 (D5) post-Th17 polarization at the indicated gene loci. Red arrows indicate regions of increased Batf and H3K27me3 recruitment on D5 vs. D2 Th17 cells.

E Naÿve WT and Batf KO $CD4^{+}CD62L^{hi}$ T cells were cultured under Th0 conditions. Cells were collected on day 5 and used to assess Stat5 binding at the indicated genomic regions by ChIP–qPCR.

Data information: Data are representative of two independent experiments with similar results (A-D) or mean ± s.e.m. of 3 independent experiments (E). (*$P < 0.05$; two-sided *t*-test).

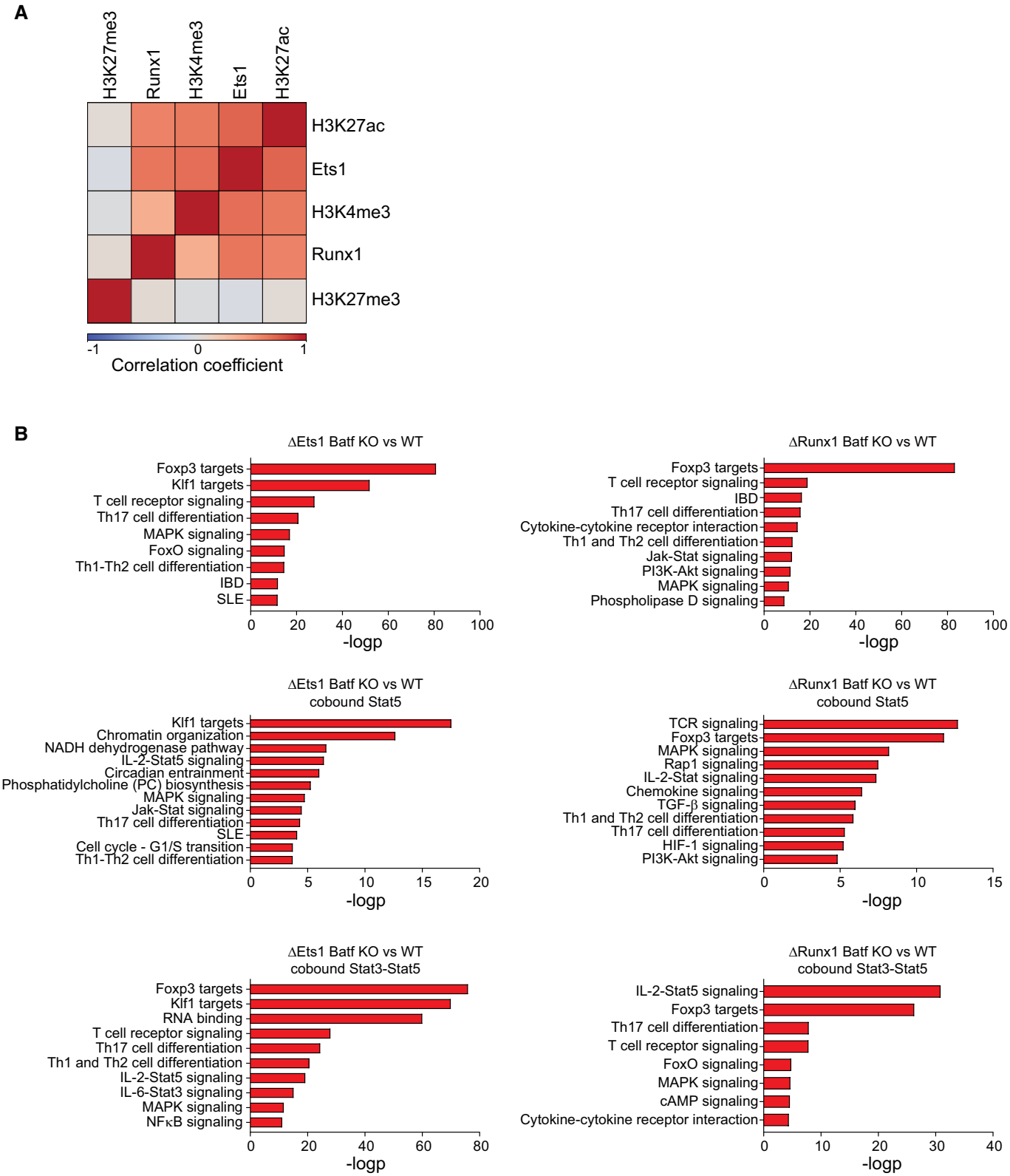

Figure EV4.

Figure EV4. Ets1 and Runx1 occupancy associates with active histone modification marks and Foxp3 targets and IL-2–Stat5 pathway in the absence of Batf.

A Naÿve WT $CD4^+CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions for 5 days and recovered cells used to perform ChIP-seq using antibodies to Batf, Ets1, Runx1, H3K4me3, H3K27me3, and H3K27ac. Stat3 and Stat5 ChIP-seq were performed after IL-6 and IL-2 stimulation, respectively, using day 5 WT Th17 cells. Heatmap shows Pearson correlation in transcription factor binding (Ets1 and Runx1) and chromatin states (H3K4me3, H3K27me3, and H3K27ac) in WT Th17 cells.

B Naÿve WT and Batf KO $CD4^+CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions for 5 days and recovered cells used to perform ChIP-seq using antibodies to Ets1 and Runx1. Stat3 and Stat5 ChIP-seq were performed after IL-6 and IL-2 stimulation, respectively. Differential enriched Ets1 (ΔEts1 Batf KO/WT) and Runx1 (ΔRunx1 Batf KO/WT) peaks in Batf KO compared with WT cells that co-bound either Stat5 or Stat3-Stat5 sites were used for genomic annotation analysis.

Figure EV5. Stat5 inhibition enhances Batf, Stat3, and p300 binding to the *Il17*a locus.

A–C Naÿve WT and Batf KO $CD4^+CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions with or without the addition of 1 μM Stat5 inhibitor. On day 3, cells were harvested and stained intracellularly for flow cytometric analysis of phosphorylated Stat5 (pStat5) to validate the effect of Stat5 inhibitor (A). Day 5 of differentiated WT and Batf KO cells were restimulated with PMA and ionomycin for 5 h and stained intracellularly for IFN-γ and IL-17a production represented by plots with percentage ± s.e.m. of positive cells (B). On day 5, cells were harvested and used to perform ChIP–qPCR using antibodies to Batf, Stat3, p300, and Stat5 (C).

D–G Naÿve WT and Stat5 cKO $CD4^+CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions. Differentiated Th17 cells were reactivated with anti-CD3 for 24 h to measure cytokine production by ELISA (D), restimulated with PMA and ionomycin for 5 h, and stained intracellularly for IFN-γ and IL-17a production represented by plots with percentage ± s.e.m. of positive cells (E), used for total RNA isolation to assess gene expression by RT–PCR (F), or used to performed ChIP-seq to assess Ets1 binding at the indicated gene loci (G). Blue arrows indicate regions of increased Ets1 recruitment on WT vs. Stat5 cKO cells.

Data information: Data are mean ± s.e.m. of three independent experiments (A–F) or representative of two independent experiments with similar results (G) (*$P < 0.05$; two-sided t-test or one-way ANOVA followed by Tukey's test).

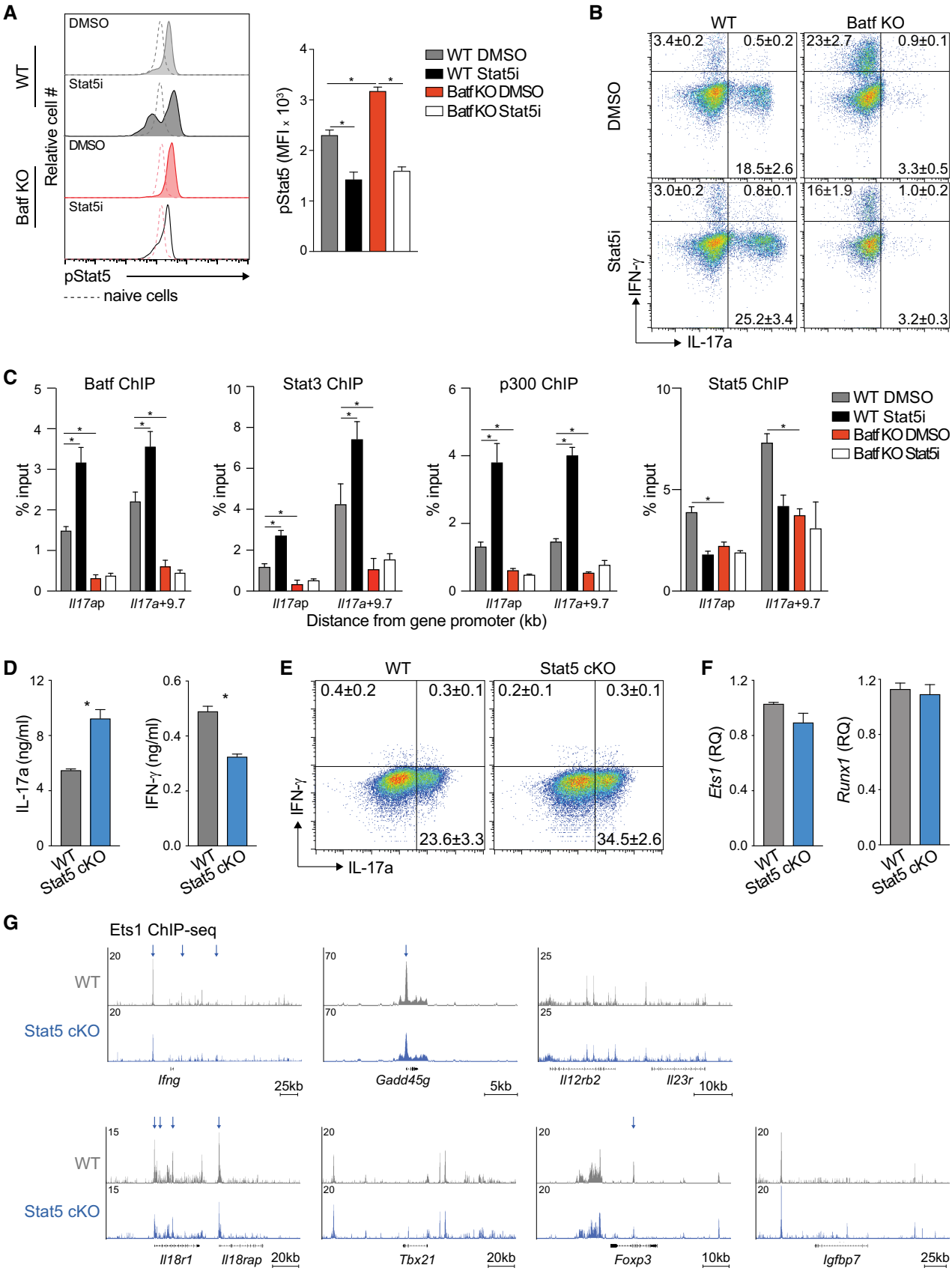

Figure EV5.

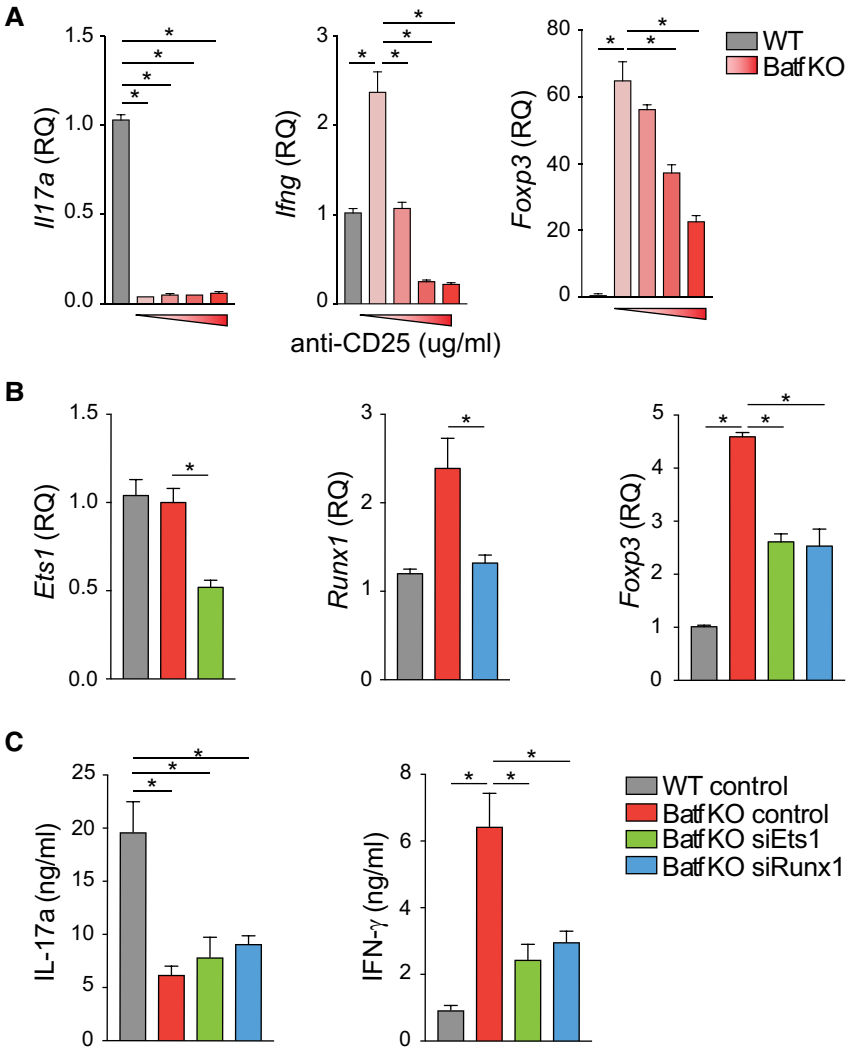

Figure EV6. Ets1 and Runx1 regulate *Ifng*/IFN-γ and *Foxp3* expression in Batf-deficient Th17-polarized cells.

A Naÿve Batf KO T cells were cultured under Th17-polarizing conditions with the addition of 0, 1, 5, or 10 μg/ml anti-CD25 antibody (red bars). Untreated WT cells (gray bars) served as a control. On day 5, cells were harvested for total RNA isolation to assess gene expression by RT–PCR (*Foxp3*, no restimulation; *Ifng* and *Il17a*, restimulated with 2 μg/ml anti-CD3 for 6 h). Data are normalized to untreated WT Th17 cells.

B, C Naÿve WT and Batf KO $CD4^{+}CD62L^{hi}$ T cells were cultured under Th17-polarizing conditions. On day 3, cells were transfected with control or siRNA targeting Ets1 or Runx1, and cultured an additional 24 h without restimulation prior to total RNA isolation to assess gene expression (B; data normalized to WT control), or with the addition of 2 μg/ml anti-CD3 for measurement of cytokine production by ELISA (C).

Data information: Data are mean ± s.e.m. of three independent experiments. (*$P < 0.05$; one-way ANOVA followed by Tukey's test).